TruSight® Tumor 170

A comprehensive next-generation sequencing assay that targets DNA and RNA variants from the same formalin-fixed, paraffin-embedded (FFPE) tumor sample.

Highlights

- **Comprehensive Coverage of Cancer-Related Variants**
  Single-assay efficiency using DNA and RNA for assessment of small variants, amplifications, splice variants, and fusions
- **Integrated, Streamlined Workflow**
  DNA and RNA libraries are prepared in parallel with an integrated workflow following DNA shearing/cDNA synthesis
- **Accurate Results from Low-Quality Samples**
  Variant detection with 40 ng DNA/RNA input, and as low as 5% mutant allele frequency, from FFPE samples

Introduction

Cancer is a leading cause of death worldwide and has the potential to originate in any tissue.[1] Analyzing the genetic basis of a given tumor is important for understanding its progression and developing new methods of treatment. However, numerous genes can cause or influence tumor progression, and many heterogeneous tumors carry multiple mutations. Furthermore, the function of any gene can be altered by several types of variations including single nucleotide variants (SNVs), multiple nucleotide variants (MNVs), small insertions or deletions (indels), amplifications, splice variations, and gene fusions. Therefore, it is difficult for researchers to analyze tumors efficiently when available methods only cover a portion of these variations, and sequential testing consumes valuable tissue, time, and resources.

To help researchers address this challenge, Illumina offers TruSight Tumor 170, a next-generation sequencing (NGS) assay designed to cover 170 genes associated with solid tumors. TruSight Tumor 170 is an enrichment-based targeted panel that simultaneously analyzes DNA and RNA, covering a wide range of genes and variant types. The panel is designed to work with the NextSeq™ 500, NextSeq 550, or HiSeq™ 2500 Sequencing Systems (Figure 1).

Comprehensive Cancer-Related Content Design

TruSight Tumor 170 targets all coding exons, per the current RefSeq database,[2] in 170 genes (Table 1). The genes and type of variant analysis for each gene were carefully selected to include content cited by professional organizations such as the National Comprehensive Cancer Network (NCCN) and the European Society for Medical Oncology (ESMO).[3,4] Independent consortia publications and late-stage pharmaceutical research also influenced the design of TruSight Tumor 170. The content includes 55 genes for fusions and splice variants, 148 SNVs and indels, and 59 amplifications. By harnessing the expertise of recognized authorities in the oncology community, TruSight Tumor 170 provides researchers with comprehensive coverage of the variants that are most likely to play a role in tumorigenesis.

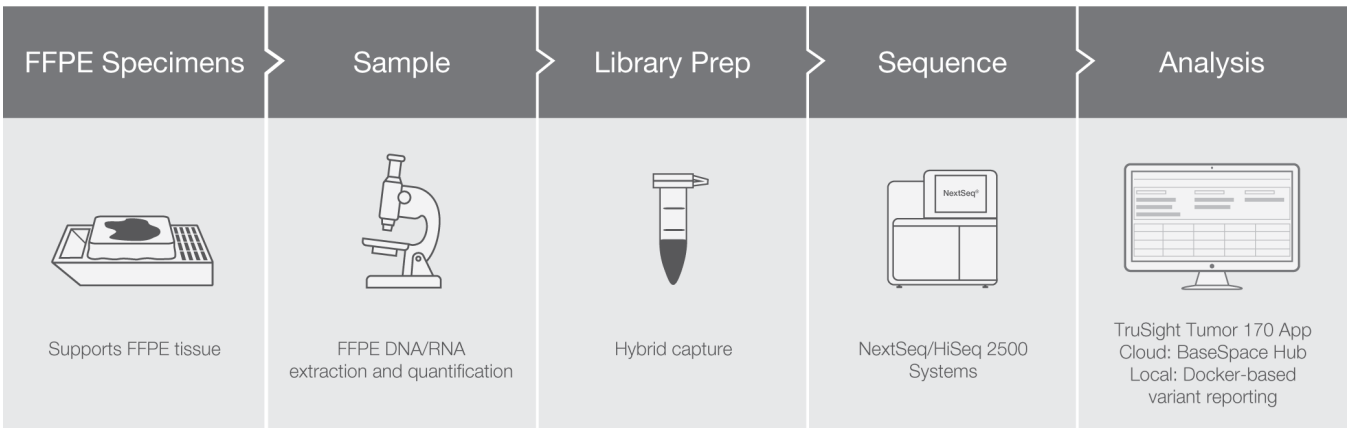

Figure 1: TruSight Tumor 170 Workflow—TruSight Tumor 170 is optimized for integration into current lab workflows, going from extracted nucleic acids to variant calling in less than 4 days. The assay can be run on the NextSeq Series or HiSeq 2500 System.

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Table 1: Gene Content in the TruSight Tumor 170 Assay

SNVs and Indels (from DNA)									
AKT1	*BRIP1*	*CREBBP*	*FANCI*	*FGFR2*	*JAK3*	*MSH3*	*PALB2*	*RAD51D*	*TSC1*
AKT2	*BTK*	*CSF1R*	*FANCL*	*FGFR3*	*KDR*	*MSH6*	*PDGFRA*	*RAD54L*	*TSC2*
AKT3	*CARD11*	*CTNNB1*	*FBXW7*	*FGFR4*	*KIT*	*MTOR*	*PDGFRB*	*RB1*	*VHL*
ALK	*CCND1*	*DDR2*	*FGF1*	*FLT1*	*KMT2A (MLL)*	*MUTYH*	*PIK3CA*	*RET*	*XRCC2*
APC	*CCND2*	*DNMT3A*	*FGF2*	*FLT3*	*KRAS*	*MYC*	*PIK3CB*	*RICTOR*	
AR	*CCNE1*	*EGFR*	*FGF3*	*FOXL2*	*MAP2K1*	*MYCL1*	*PIK3CD*	*ROS1*	
ARID1A	*CD79A*	*EP300*	*FGF4*	*GEN1*	*MAP2K2*	*MYCN*	*PIK3CG*	*RPS6KB1*	
ATM	*CD79B*	*ERBB2*	*FGF5*	*GNA11*	*MCL1*	*MYD88*	*PIK3R1*	*SLX4*	
ATR	*CDH1*	*ERBB3*	*FGF6*	*GNAQ*	*MDM2*	*NBN*	*PMS2*	*SMAD4*	
BAP1	*CDK12*	*ERBB4*	*FGF7*	*GNAS*	*MDM4*	*NF1*	*PPP2R2A*	*SMARCB1*	
BARD1	*CDK4*	*ERCC1*	*FGF8*	*HNF1A*	*MET*	*NOTCH1*	*PTCH1*	*SMO*	
BCL2	*CDK6*	*ERCC2*	*FGF9*	*HRAS*	*MLH1*	*NOTCH2*	*PTEN*	*SRC*	
BCL6	*CDKN2A*	*ERG*	*FGF10*	*IDH1*	*MLLT3*	*NOTCH3*	*PTPN11*	*STK11*	
BRAF	*CEBPA*	*ESR1*	*FGF14*	*IDH2*	*MPL*	*NPM1*	*RAD51*	*TERT*	
BRCA1	*CHEK1*	*EZH2*	*FGF23*	*INPP4B*	*MRE11A*	*NRAS*	*RAD51B*	*TET2*	
BRCA2	*CHEK2*	*FAM175A*	*FGFR1*	*JAK2*	*MSH2*	*NRG1*	*RAD51C*	*TP53*	
Amplifications (from DNA)									
AKT2	*BRCA2*	*CHEK1*	*ERCC2*	*FGF5*	*FGF14*	*FGFR4*	*MDM4*	*NRG1*	*RAF1*
ALK	*CCND1*	*CHEK2*	*ESR1*	*FGF6*	*FGF19*	*JAK2*	*MET*	*PDGFRA*	*RET*
AR	*CCND3*	*EGFR*	*FGF1*	*FGF7*	*FGF23*	*KIT*	*MYC*	*PDGFRB*	*RICTOR*
ATM	*CCNE1*	*ERBB2*	*FGF2*	*FGF8*	*FGFR1*	*KRAS*	*MYCL1*	*PIK3CA*	*RPS6KB1*
BRAF	*CDK4*	*ERBB3*	*FGF3*	*FGF9*	*FGFR2*	*LAMP1*	*MYCN*	*PIK3CB*	*TFRC*
BRCA1	*CDK6*	*ERCC1*	*FGF4*	*FGF10*	*FGFR3*	*MDM2*	*NRAS*	*PTEN*	
Fusions and Splice Variants (from RNA)									
ABL1	*BRAF*	*EML4*	*ETV4*	*FGFR4*	*KIF5B*	*MYC*	*NTRK2*	*PIK3CA*	*TMPRSS2*
AKT3	*BRCA1*	*ERBB2*	*ETV5*	*FLI1*	*KIT*	*NOTCH1*	*NTRK3*	*PPARG*	
ALK	*BRCA2*	*ERG*	*EWSR1*	*FLT1*	*KMT2A (MLL)*	*NOTCH2*	*PAX3*	*RAF1*	
AR	*CDK4*	*ESR1*	*FGFR1*	*FLT3*	*MET*	*NOTCH3*	*PAX7*	*RET*	
AXL	*CSF1R*	*ETS1*	*FGFR2*	*JAK2*	*MLLT3*	*NRG1*	*PDGFRA*	*ROS1*	
BCL2	*EGFR*	*ETV1*	*FGFR3*	*KDR*	*MSH2*	*NTRK1*	*PDGFRB*	*RPS6KB1*	

Combined Workflow for DNA and RNA

Library preparation for TruSight Tumor 170 uses an enrichment method that can be simultaneously applied to DNA and RNA extracted from the same sample. After the initial steps, in which genomic DNA is sheared and RNA is converted to cDNA, library prep becomes a combined workflow (Figure 2).

- Sheared DNA and cDNA are converted into sequenceable libraries.
- Regions of interest are hybridized to biotinylated probes, magnetically pulled down with streptavidin-coated beads, and eluted to enrich the library pool.
- Libraries are normalized using a simple bead-based protocol before pooling and sequencing.

TruSight Tumor 170 Data Analysis

Illumina sequencing systems offer the option to connect to BaseSpace® Sequence Hub, the Illumina genomics computing environment for sequencing data analysis and management. Researchers can securely store, analyze, archive, and share sequencing data. The TruSight Tumor 170 App is designed to make variant calls that enable downstream reporting in an easy-to-read format. Raw data outputs for small variants, amplifications, fusions, and splice variants are provided, as well as user-friendly, focused outputs for high confidence RNA variants and fusion results.

The TruSight Tumor 170 App is available in BaseSpace Sequence Hub. For users who desire locally based secondary analysis, Illumina offers a Docker-based image of the app. Contact your sales or support representative for further information.

Sensitive, Highly Confident Variant Detection

Deep sequencing using NGS provides the high sensitivity to reveal somatic variation in tumor subpopulations. Illumina sequencing by synthesis (SBS) chemistry is the most widely adopted NGS technology, generating > 90% of global sequencing data.* When paired with high-quality sequencing on the NextSeq and HiSeq Systems, TruSight Tumor 170 provides uniform coverage of target regions, identifying somatic mutations as low as 5% mutant allele frequency with ≥ 250× minimum coverage (Table 2).

* Data Calculations on file, Illumina, Inc., 2015.

Figure 2: Combined Library Prep Workflow—The DNA and RNA samples follow the same workflow, after the cDNA synthesis step (for RNA) and the shearing step (for DNA).

Table 2: Specifications

Parameter	Details
System	NextSeq or HiSeq 2500 System
Panel Size	533 kb DNA 358 kb RNA
Minimum Insert Size	79 bp DNA 63 bp RNA
DNA Input Requirement	40 ng total
RNA Input Requirement	40 ng total
Library Preparation Time	32 hours
Sequence Run Time	24 hours (NextSeq Systems) or 27 hours (HiSeq 2500 System)
Sequence Run	2 × 101 cycles
Kit Size	24 samples (both DNA and RNA)
Sample Throughput	8 samples per run (NextSeq Systems) or 6 samples per rapid run (HiSeq 2500 System)
Sensitivity	5% Mutant Allele Frequency > 95% sensitivity and specificity

High Coverage of Targets from Low-Quality Samples

Nucleic acids extracted from FFPE tissues have the potential to fail quality control checks and yield poor target coverage resulting in low analytical sensitivity. TruSight Tumor 170 addresses this issue by generating libraries from nucleic acids of small fragment size, as low as 79 bp for DNA and 63 bp for RNA. This enables deep coverage of FFPE samples, even when the quality of extracted nucleic acids is low (Figure 3).

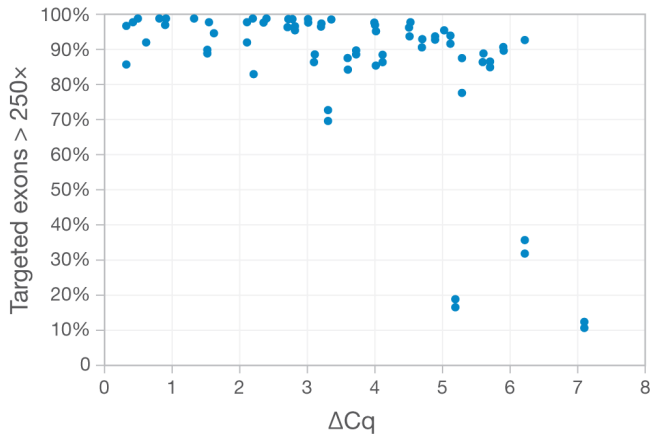

Figure 3: Target Coverage from FFPE Samples—DNA from FFPE tumor samples of varying quality was extracted and evaluated using the TruSight Tumor 170 assay and sequenced on the NextSeq 500 System. Quality of each sample was also assessed using qPCR to measure DNA amplification potential. The ΔCq value indicates the cycle threshold (Ct) value of each DNA sample minus the Ct value of a DNA standard.

Reliable Small Variant Detection From High and Low-Quality Samples

TruSight Tumor 170 provides sensitivity and accuracy for identifying low-frequency variations in samples of varying quality. High target coverage enables confident calling of low-level variants in characterized cell lines (Table 3). TruSight Tumor 170 enables variant detection in FFPE tumor samples with as low as 5% mutant allele frequency (Table 4).

Table 3: Small Variant Calling with Characterized Cell Lines

Gene	Mutation	Reported Frequency	Detected Frequency	Coverage
APC	R2714C	0.33	0.31	2547×
ARID1A	P1562fs	0.34	0.31	419×
BRAF	V600E	0.10	0.11	2282×
BRCA2	A1689fs	0.33	0.30	1097×
EGFR	G719S	0.24	0.22	2207×
EP300	K291fs	0.08	0.06	1359×
FBXW7	G667fs	0.34	0.30	2870×
FGFR1	P150L	0.08	0.08	1102×
FLT3	S985fs	0.10	0.10	1925×
FLT3	V197A	0.12	0.10	1908×
IDH1	S261L	0.10	0.09	2052×
KIT	D816V	0.10	0.15	1239×
KRAS	G13D	0.15	0.14	1507×
KRAS	G12D	0.06	0.07	1503×
MET	V237fs	0.06	0.06	3700×
MLH1	L323M	0.08	0.09	1725×
NF1	L626fs	0.08	0.10	1270×
NOTCH1	P668S	0.32	0.32	1637×
NRAS	Q61K	0.12	0.14	1824×
PDGFRA	G426D	0.34	0.29	2018×
PI3KCA	E545K	0.09	0.16	773×
PI3KCA	H1047R	0.18	0.15	1694×

DNA from the HD200, a formalin-fixed cell line (Horizon Diagnostics) containing known variants, was evaluated using the TruSight Tumor 170 assay and sequenced on the NextSeq 500 System. 100% concordance was observed with expected frequency from all HD200 variants.

Table 4: Small Variant Detection with FFPE Tumor Samples

Sample	Reported Mutation	Detected Mutation	Detected Frequency	Coverage
FFPE_Colon	*TP53* R158C	*TP53* R158C	0.057	1545×
FFPE_Bone	*TP53* P72R	*TP53* P72R	0.059	515×
FFPE_Brain1	*PIK3CA* E545G	*PIK3CA* E545G	0.078	289×
FFPE_Brain2	*PIK3CA* H1047R	*PIK3CA* H1047R	0.076	531×
FFPE_Breast	*KRAS* G12D	*KRAS* G12D	0.049	1671×
FFPE_Lung1	*KRAS* G12D	*KRAS* G12D	0.059	575×
FFPE_Lung2	*TP53* C242F	*TP53* C242F	0.080	691×
FFPE_Skin	*TP53* R248Q	*TP53* R248Q	0.050	1240×

DNA from FFPE tumor samples was extracted and evaluated using the TruSight Tumor 170 assay and sequenced on the NextSeq 500 System. All 8 FFPE samples had 100% concordance with reported mutations.

Reliable Calling of Amplifications, Fusions, and Splice Variants from FFPE Samples

TruSight Tumor 170 combines the sensitivity of Illumina sequencing systems with new software platforms to enable simultaneous calling of amplifications, fusions, and splice variants. The TruSight Tumor 170 App features novel variant calling algorithms that produce accurate calls for splice variants, fusions, and gene amplifications from raw sequencing data in samples of varying quality (Tables 5 and 6).

Table 5: Amplification Calling with FFPE Tumor Samples

Sample	Reported Amplification	Reported Amplification Level	Detected Amplification	Detected Amplification Level
FFPE_Bone	*FGF19*	1.4	*FGF19*	2.9
FFPE_Brain2	*PDGFRA*	2.3	*PDGFRA*	2.9
FFPE_Breast	*RPS6KB1*	2.4	*RPS6KB1*	2.4
FFPE_Colon	*BRCA2*	2.2	*BRCA2*	2.0
FFPE_Lung1	*PIK3CA*	2.4	*PIK3CA*	2.7
FFPE_Lung2	*FGFR1*	2.4	*FGFR1*	2.9
FFPE_Lung3	*MYC*	2.2	*MYC*	2.8
FFPE_Lung4	*CCNE1*	2.1	*CCNE1*	2.2
FFPE_Lung5	*EGFR*	2.2	*EGFR*	4.5
FFPE_Lung6	*CCND1*	2.3	*CCND1*	2.9
FFPE_Stomach1	*CDK6*	2.3	*CDK6*	1.7
FFPE_Stomach2	*MET*	1.5	*MET*	1.4

DNA from FFPE tumor samples was extracted and then evaluated using the TruSight Tumor 170 assay and sequenced on the NextSeq 500 System. All 12 FFPE samples had 100% variant concordance.

Table 6: Fusion and Splice Variants Calling with FFPE Tissues and Cell Lines

Sample	DV200	Reported Variant	Detected Variant
FFPE_Brain Tissue	N/A	EGFR VIII Splice Variant	EGFR VIII Splice Variant
FFPE_Breast Tissue	81	RPS6KB1-VMP1, RPS6KB1-DIAPH3, CCDC170-ESR1 fusions	RPS6KB1-VMP1, RPS6KB1-DIAPH3, CCDC170-ESR1 fusions
FFPE_Ewing's Tissue	48.9	EWSR1-FLI1 fusion	EWSR1-FLI1 fusion
FFPE_Gastric Cell Line	93	MET Exon 14 Skipping Splice Variant	MET Exon 14 Skipping Splice Variant
FFPE_Lung CellLine	93	CCDC6-RET fusion	CCDC6-RET fusion
FFPE_Lung Tissue1	73.3	EML4-ALK fusion	EML4-ALK fusion
FFPE_Lung Tissue2	95	FGFR3-TACC3 fusions	FGFR3-TACC3 fusions
FFPE_Prostate Cell Line	95.5	ARv7 Splice Variant	ARv7 Splice Variant
FFPE_ Prostate Tissue	28.7	TMPRSS2-ERG, TMPRSS2-GNPT fusions	TMPRSS2-ERG, TMPRSS2-GNPT fusions

RNA from FFPE tumor samples was extracted and then evaluated using the TruSight Tumor 170 assay and sequenced on the NextSeq 500 System. All 9 FFPE samples had 100% variant concordance. DV200 value is used to assess the quality of RNA used to prepare sequencing libraries, and represents the percentage of RNA fragments > 200 nucleotides.

Summary

TruSight Tumor 170 offers an integrated workflow solution for the detection of common somatic variants found in solid tumors. DNA and RNA libraries are prepared, sequenced, and analyzed simultaneously for efficient assessment of numerous types of somatic variants. Developed according to evidence-based guidelines, with input from key opinion leaders and late-stage pharmaceutical research, the panel provides labs with a comprehensive view of cancer-relevant genes and accurate analysis of low-frequency variants from FFPE DNA and RNA. By assessing 170 genes, and several different types of variants in a single assay, TruSight Tumor 170 offers a comprehensive genetic investigation of tumor samples in a streamlined solution.

Ordering Information

Library Prep Kits	No. of Samples	Catalog No.
TruSight Tumor 170 NextSeq Kit	24	OP-101-1003
TruSight Tumor 170 Kit	24	OP-101-1004

Learn More

For more information about TruSight Tumor 170, visit www.illumina.com/TruSightTumor170

References

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